Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=OR			
L27	L26 and (angioplasty or stent)	219	L27
L26	(l1 same cancer) and (taxol or taxane or paclitaxel)	436	L26
L25	l1 same cancer same (taxol or taxane or paclitaxel)	18	L25
L24	L23 and (taxol or taxane or paclitaxel)	9	L24
L23	l22 same cancer	28	L23
L22	neointimal near hyperplasia	551	L22
L21	5498421.pn. and cancer	1	L21
L20	L19 and (angioplasty or stent)	151	L20
L19	l16 and paclitaxel	251	L19
L18	L17 and (protein near coat$)	0	L18
L17	L16 and l9	0	L17
L16	cancer same l3	3755	L16
L15	cancer same l2	0	L15
L14	cancer same l1	3673	L14
L13	L9 and (angioplasty or stent)	7	L13
L12	L9 and cancer	14	L12
DB=USPT; PLUR=YES; OP=OR			
L11	5270052.pn.	1	L11
DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=OR			
L10	L9 and l1	1	L10
L9	protein near coat$ near (drug or taxane or paclitaxel or active)	19	L9
L8	L6 and (protein near coat$)	2	L8
L7	L6 and (drug same protein)	18	L7
L6	L5 and (angioplasty or stent)	45	L6
L5	L4 and paclitaxel	50	L5
L4	neointimal near hyperplasia	551	L4
L3	neointimal hyperplasia	11005	L3
L2	neointimalhyperplasia	0	L2
L1	hyperplasia	10491	L1

END OF SEARCH HISTORY

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L27: Entry 215 of 219 File: USPT Mar 31, 1998

DOCUMENT-IDENTIFIER: US 5733925 A
**** See image for Certificate of Correction ****
TITLE: Therapeutic inhibitor of vascular smooth muscle cells

Abstract Text (1):
Methods are provided for inhibiting stenosis following vascular trauma or disease in a mammalian host, comprising administering to the host a therapeutically effective dosage of a therapeutic conjugate containing a vascular smooth muscle binding protein that associates in a specific manner with a cell surface of the vascular smooth muscle cell, coupled to a therapeutic agent dosage form that inhibits a cellular activity of the muscle cell. Methods are also provided for the direct and/or targeted delivery of therapeutic agents to vascular smooth muscle cells that cause a dilation and fixation of the vascular lumen by inhibiting smooth muscle cell contraction, thereby constituting a biological stent. Also discussed are mechanisms for in vivo vascular smooth muscle cell proliferation modulation, agents that impact those mechanisms and protocols for the use of those agents.

Brief Summary Text (2):
This invention relates generally to therapeutic methods involving surgical or intravenous introduction of binding partners directed to certain target cell populations, such as smooth muscle cells, cancer cells, somatic cells requiring modulation to ameliorate a disease state and effector cells of the immune system, particularly for treating conditions such as stenosis following vascular trauma or disease, cancer, diseases resulting from hyperactivity or hyperplasia of somatic cells and diseases that are mediated by immune system effector cells. Surgical or intravenous introduction of active agents capable of altering the proliferation or migration of smooth muscle cells or contraction of smooth muscle proteins is also described. The invention also relates to the direct or targeted delivery of therapeutic agents to vascular smooth muscle cells that results in dilation and fixation of the vascular lumen (biological stenting effect). Combined administration of a cytocidal conjugate and a sustained release dosage form of a vascular smooth muscle cell inhibitor is also disclosed. Mechanisms for in vivo vascular smooth muscle cell proliferation modulation, agents that impact those mechanisms and protocols for the use of those agents are discussed.

Brief Summary Text (4):
Percutaneous transluminal coronary angioplasty (PTCA) is widely used as the primary treatment modality in many patients with coronary artery disease. PTCA can relieve myocardial ischemia in patients with coronary artery disease by reducing lumen obstruction and improving coronary flow. The use of this surgical procedure has grown rapidly, with 39,000 procedures performed in 1983, nearly 150,000 in 1987, 200,000 in 1988, 250,000 in 1989, and over 500,000 PTCAs per year are estimated by 1994 (1, 2, 3). Stenosis following PTCA remains a significant problem, with from 25% to 35% of the patients developing restenosis within 1 to 3 months. Restenosis results in significant morbidity and mortality and frequently necessitates further interventions such as repeat angioplasty or coronary bypass surgery. No surgical intervention or post-surgical treatment (to date) has proven effective in preventing restenosis.

Brief Summary Text (6):
Compounds that reportedly suppress smooth muscle proliferation in vitro (4, 6, 7) may have undesirable pharmacological side effects when used in vivo. Heparin is an example of one such compound, which reportedly inhibits smooth muscle cell

proliferation in vitro but when used in vivo has the potential adverse side effect of inhibiting coagulation. Heparin peptides, while having reduced anti-coagulant activity, have the undesirable pharmacological property of having a short pharmacological half-life. Attempts have been made to solve such problems by using a double balloon catheter, i.e., for regional delivery of the therapeutic agent at the angioplasty site (e.g., 8; U.S. Pat. No. 4,824,436), and by using biodegradable materials impregnated with a drug, i.e., to compensate for problems of short half-life (e.g., 9; U.S. Pat. No. 4,929,602).

Brief Summary Text (11):
In one aspect of the invention, new therapeutic methods and therapeutic conjugates are provided for inhibiting vascular smooth muscle cells in a mammalian host. The therapeutic conjugates contain a vascular smooth muscle binding protein or peptide that binds in a specific manner to the cell membranes of a vascular smooth muscle cell or an interstitial matrix binding protein/peptide that binds in a specific manner to interstitial matrix (e.g., collagen) of the artery wall, coupled to a therapeutic agent that inhibits the activity of the cell. In one embodiment, inhibition of cellular activity results in reducing, delaying, or eliminating stenosis after angioplasty or other vascular surgical procedures. The therapeutic conjugates of the invention achieve these advantageous effects by associating with vascular smooth muscle cells and pericytes, which may transform into smooth muscle cells. The therapeutic conjugate may contain: (1) therapeutic agents that alter cellular metabolism or are inhibitors of protein synthesis, cellular proliferation, or cell migration; (2) microtubule and microfilament inhibitors that affect morphology or increases in cell volume; and/or (3) inhibitors of extracellular matrix synthesis or secretion. In one representative embodiment, the conjugates include a cytotoxic therapeutic agent that is a sesquiterpenoid mycotoxin such as a verrucarin or a roridin. Other embodiments involve cytostatic therapeutic agents that inhibit DNA synthesis and proliferation at doses that have a minimal effect on protein synthesis such as protein kinase inhibitors (e.g., staurosporin), suramin, transforming growth factor-beta (TGF-beta) activators or production stimulators such as trans-2-[4-(1,2-diphenyl-1-butenyl)phenoxy]-N,N-dimethylethylamine (tamoxifen), TGF-beta itself, and nitric oxide releasing compounds (e.g., nitroglycerin) or analogs or functional equivalents thereof. Other moieties that inhibit cell division and are, therefore, useful in the practice of the present invention, include, for example, taxol and analogs thereof such as taxotere. In addition, therapeutic agents that inhibit the contraction or migration of smooth muscle cells and maintain an enlarged luminal area following, for example, angioplasty trauma (e.g., the cytochalasins, such as cytochalasin B, cytochalasin C, cytochalasin D, taxol or analogs thereof such as taxotere or the like) are also contemplated for use in accordance with the present invention. Other aspects of the invention relate to vascular smooth muscle binding proteins that specifically associate with a chondroitin sulfate proteoglycan (CSPG) expressed on the membranes of a vascular smooth muscle cell, and in a preferred embodiment this CSPG has a molecular weight of about 250 kDaltons. In preferred embodiments the vascular smooth muscle binding protein binds to a CSPG target on the cell surface with an association constant of at least 10.sup.-4 M. In another preferred embodiment, the vascular smooth muscle binding protein contains a sequence of amino acids found in the Fab, Fv or CDR (complementarity determining regions) of monoclonal antibody NR-AN-01 or functional equivalents thereof.

Brief Summary Text (12):
Other aspects of the invention include methods for inhibiting stenosis, e.g., following angioplasty in a mammalian host, by administering to a human or animal subject in need of such treatment a therapeutically effective dosage of a therapeutic conjugate of the invention. In one representative embodiment, the dosage of therapeutic conjugate may be administered with an infusion catheter, to achieve a 10.sup.-3 M to 10.sup.-12 M concentration of said therapeutic conjugate at the site of administration in a blood vessel.

Brief Summary Text (15):
Preferable therapeutic agents dispersed within the microparticulates or nanoparticulates are those exhibiting inhibition of a therapeutically significant target cell activity without killing the target cell, or target cell killing activity. For treatment of restenosis of vascular smooth muscle cells, useful

therapeutic agents inhibit target cell activity (e.g., proliferation or migration) without killing the target cells. Preferred therapeutic moieties for this purpose are protein kinase inhibitors (e.g., staurosporin or the like), TGF-beta production or activation stimulators, such as tamoxifen or TGF-beta itself, taxol or analogs thereof (e.g., taxotere), smooth muscle migration and/or contraction inhibitors (e.g., the cytochalasins, such as cytochalasin B, cytochalasin C, cytochalasin D or the like), suramin, and nitric oxide- releasing compounds, such as nitroglycerin, or analogs or functional equivalents thereof. In cancer therapy, useful therapeutic agents inhibit proliferation or are cytotoxic to the target cells. Preferred therapeutic moieties for this purpose are TGF-beta production or activation stimulators, such as tamoxifen or TGF-beta itself, taxol or analogs thereof (e.g., taxotere), Roridin A and Pseudomonas exotoxin, or analogs or functional equivalents thereof. For treatment of immune system-modulated diseases, such as arthritis, useful therapeutic agents deliver cytostatic, cytocidal or metabolism-modulating therapeutic agents to target cells that are accessible by local administration of the dosage form. Preferred therapeutic moieties for this purpose are Roridin A, Pseudomonas exotoxin, suramin, TGF-beta production or activation stimulators, such as tamoxifen or TGF-beta itself, taxol or analogs thereof (e.g., taxotere) and protein kinase inhibitors (e.g., staurosporin), sphingosine, or analogs or functional equivalents thereof. For treatment of pathologically proliferating normal tissues (e.g., proliferative vitreoretinopathy, corneal pannus and the like), anti-proliferative agents or antimigration agents are preferred (e.g., cytochalasins, taxol or analogs thereof, somatostatin, somatostatin analogs, N-ethylmaleimide, antisense oligonucleotides, TGF-beta production or activation stimulators, such as tamoxifen or TGF-beta itself and the like).

Brief Summary Text (17):
The present invention also provides therapeutic methods and therapeutic dosage forms involving administration of free (i.e., non-targeted or non-binding partner associated) therapeutic agent to target cells. Preferably, the target cells are vascular smooth muscle cells and the therapeutic agent is an inhibitor of vascular smooth muscle cell contraction, allowing the normal hydrostatic pressure to dilate the vascular lumen. Such contraction inhibition may be achieved by actin inhibition, which is preferably achievable and sustainable at a lower dose level than that necessary to inhibit protein synthesis. Consequently, the vascular smooth muscle cells synthesize protein required to repair minor cell trauma and secrete interstitial matrix, thereby facilitating the fixation of the vascular lumen in a dilated state near its maximal systolic diameter. This phenomenon constitutes a biological stenting effect that diminishes or prevents the undesirable recoil mechanism that occurs in up to 25% of the angioplasty procedures classified as successful based on an initial post-procedural angiogram. Cytochalasins (which inhibit the polymerization of G- to F-actin which, in turn, inhibits the migration and contraction of vascular smooth muscle cells) are the preferred therapeutic agents for use in this embodiment of the present invention. Free therapeutic agent protocols of this type effect a reduction, a delay, or an elimination of stenosis after angioplasty or other vascular surgical procedures. Preferably, free therapeutic agent is administered directly or substantially directly to vascular smooth muscle tissue. Such administration is preferably effected by an infusion catheter, to achieve a 10.sup.-3 M to 10.sup.-12 M concentration of said therapeutic agent at the site of administration in a blood vessel.

Brief Summary Text (19):
TGF-beta, TGF-beta activator and TGF-beta production stimulator sustained release dosage forms of the present invention may be employed in the prevention or treatment of conditions characterized by inappropriate proliferation of smooth muscle cells, such as the prevention or reduction of restenosis following angioplasty or other vascular trauma. TGF-beta or such TGF-beta activators and production stimulators inhibit abnormal proliferation of smooth muscle cells. A preferred TGF-beta activator/production stimulator is trans 2-[4-(1,2-diphenyl-1-butenyl) phenoxy-N,N-dimethylethylamine.

Brief Summary Text (20):
The amount of TGF-beta, TGF-beta activator or TGF-beta production stimulator therapeutic or prophylactic agent administered in sustained release dosage forms is selected to treat vascular trauma of differing severity, with smaller doses being

sufficient to treat lesser vascular trauma such as in the prevention of vascular rejection following graft or transplant. Such dosage forms are also amenable to chronic use for prophylactic purposes with respect to disease states involving proliferation of vascular smooth muscle cells over time (e.g., atherosclerosis, coronary heart disease, thrombosis, myocardial infarction, stroke, smooth muscle neoplasms such as leiomyoma and leiomyosarcoma of the bowel and uterus, uterine fibroid or fibroma and the like). For the prevention/treatment of restenosis, for example, a large dose (optionally, in sustained release form) is administered before or during an angioplasty procedure, followed by a sustained release dosage form designed to release smaller, follow up doses over time to maintain an anti-proliferative effect for a time sufficient to substantially reduce the risk of or prevent restenosis. A preferred therapeutic protocol duration for this purpose is from about 3 to about 26 weeks.

Drawing Description Text (19):
FIG. 9A shows stenosis due to intimal smooth muscle cell proliferation in a histological section of an untreated artery 5 weeks after angioplasty in an animal model.

Drawing Description Text (20):
FIG. 9B shows inhibition of stenosis in a histological section of an artery treated with therapeutic conjugate at 5 weeks after angioplasty in an animal model.

Detailed Description Text (24):
As referred to herein, "taxol" includes taxol, analogs thereof such as taxotere as well as functional equivalents or derivatives thereof. Taxol is readily taken up into cells and stabilizes such cells against cell division.

Detailed Description Text (29):
The therapeutic conjugates and dosage forms of the invention are useful for inhibiting the activity of vascular smooth muscle cells, e.g., for reducing, delaying, or eliminating stenosis following angioplasty. As used herein the term "reducing" means decreasing the intimal thickening that results from stimulation of smooth muscle cell proliferation following angioplasty, either in an animal model or in man. "Delaying" means delaying the time until onset of visible intimal hyperplasia (e.g., observed histologically or by angiographic examination) following angioplasty and may also be accompanied by "reduced" restenosis. "Eliminating" restenosis following angioplasty means completely "reducing" and/or completely "delaying" intimal hyperplasia in a patient to an extent which makes it no longer necessary to surgically intervene, i.e., to re-establish a suitable blood flow through the vessel by repeat angioplasty, atheroectomy, or coronary artery bypass surgery. The effects of reducing, delaying, or eliminating stenosis may be determined by methods routine to those skilled in the art including, but not limited to, angiography, ultrasonic evaluation, fluoroscopic imaging, fiber optic endoscopic examination or biopsy and histology. The therapeutic conjugates of the invention achieve these advantageous effects by specifically binding to the cellular membranes of smooth muscle cells and pericytes.

Detailed Description Text (52):
Representative examples of "cytostatic agents" include, e.g., modified toxins, methotrexate, adriamycin, radionuclides (e.g., such as disclosed in Fritzberg et al., U.S. Pat. No. 4,897,255), protein kinase inhibitors (e.g., staurosporin), stimulators of the production or activation of TGF-beta, including tamoxifen and functional equivalents or derivatives thereof, TGF-beta or functional equivalents, derivatives or analogs thereof, taxol or analogs thereof (e.g., taxotere), inhibitors of specific enzymes (such as the nuclear enzyme DNA topoisomerase II and DNA polymerase, RNA polymerase, adenyl guanyl cyclase), superoxide dismutase inhibitors, terminal deoxynucleotidyl- transferase, reverse transcriptase, antisense oligonucleotides that suppress smooth muscle cell proliferation and the like, which when delivered into a cellular compartment at an appropriate dosage will act to impair proliferation of a smooth muscle cell or pericyte without killing the cell. Other examples of "cytostatic agents" include peptidic or mimetic inhibitors (i.e., antagonists, agonists, or competitive or non-competitive inhibitors) of cellular factors that may (e.g., in the presence of extracellular matrix) trigger proliferation of smooth muscle cells or pericytes: e.g., cytokines (e.g.,

interleukins such as IL-1), growth factors, (e.g., PDGF, TGF-alpha or -beta, tumor necrosis factor, smooth muscle- and endothelial-derived growth factors, i.e., endothelin, FGF), homing receptors (e.g., for platelets or leukocytes), and extracellular matrix receptors (e.g., integrins). Representative examples of useful therapeutic agents in this category of cytostatic agents for smooth muscle proliferation include: subfragments of heparin, triazolopyrimidine (Trapidil; a PDGF antagonist), lovastatin, and prostaglandins E1 or I2.

Detailed Description Text (54):
Representative examples of "cytoskeletal inhibitors" include colchicine, vinblastin, cytochalasins, taxol and the like that act on microtubule and microfilament networks within a cell.

Detailed Description Text (58):
Representative examples of "anti-matrix agents" include inhibitors (i.e., agonists and antagonists and competitive and non-competitive inhibitors) of matrix synthesis, secretion and assembly, organizational cross-linking (e.g., transglutaminases cross-linking collagen), and matrix remodeling (e.g., following wound healing). A representative example of a useful therapeutic agent in this category of anti-matrix agents is colchicine, an inhibitor of secretion of extracellular matrix. Another example is tamoxifen for which evidence exists regarding its capability to organize and/or stabilize as well as diminish smooth muscle cell proliferation following angioplasty. The organization or stabilization may stem from the blockage of vascular smooth muscle cell maturation in to a pathologically proliferating form.

Detailed Description Text (59):
For the sustained release dosage form embodiments of the present invention, therapeutic agents preferably are those that inhibit vascular smooth muscle cell activity without killing the cells (i.e., cytostatic therapeutic agents). Another way to define a cytostatic agent is a moiety capable of inhibiting one or more pathological activities of the target cells for a time sufficient to achieve a therapeutic benefit. Preferred therapeutic agents for this purpose exhibit one or more of the following capabilities: to inhibit DNA synthesis prior to protein synthesis inhibition or to inhibit migration of vascular smooth muscle cells into the intima. These therapeutic agents do not significantly inhibit protein synthesis (i.e., do not kill the target cells) and, therefore, facilitate cellular repair and matrix production to stabilize the vascular wall lesion caused by angioplasty, by reducing smooth muscle cell proliferation.

Detailed Description Text (60):
Exemplary of such preferred therapeutic agents are protein kinase inhibitors, such as staurosporin (staurosporine is available from Sigma Chemical Co., St. Louis, Mo.) cytochalasins, such as cytochalasin B (Sigma Chemical Co.), and suramin (FBA Pharmaceuticals, West Haven, Conn.), as well as nitroglycerin (DuPont Pharmaceuticals, Inc., Manuti, Puerto Rico) or analogs or functional equivalents thereof. These compounds are cytostatic and have been shown to exert minimal protein synthesis inhibition and cytotoxicity at concentrations where significant DNA synthesis inhibition occurs (see Example 8 and FIGS. 10A-10D). Other exemplary preferred therapeutic agents are TGF-beta activators or production stimulators, such as tamoxifen and functional equivalents or derivatives thereof. TGF-beta and its analogs, derivatives or functional equivalents may also be employed. Taxol and its analogs, derivatives or functional equivalents are also useful in the practice of the present invention. A useful protocol for identifying therapeutic agents useful in sustained release dosage form embodiments of the present invention is set forth in Example 8, for example. A practitioner in the art is capable of designing substantially equivalent experimental protocols for making such an identification for different target cell populations, such as adherent monolayer target cell types.

Detailed Description Text (62):
Modulation of immune system-mediated disease effector cells can also be accomplished using the sustained release dosage forms of the present invention. Such modulation is preferably conducted with respect to diseases having an effector cell population that is accessible through local sustained release dosage form administration. Therapeutic moieties having the requisite modulating activity, e.g., cytocidal,

cytostatic, metabolism modulation or like activity upon lymphorecticular cells in the treatment of arthritis (intra-articular administration), sprue (oral administration), uveitis and endophthalmitis (intra-ocular administration) and keratitis (sub-conjunctival administration), are identifiable using techniques that are known in the art. These agents can also be used to reduce hyperactivity of epithelial glands and endocrine organs that results in multiple disorders. Preferred agents for these embodiments include Roridin A, Pseudomonas exotoxin, suramin, protein kinase inhibitors (e.g., staurosporin), TGF-beta and TGF-beta activators or production stimulators such as tamoxifen, taxol and the like, or analogs or functional equivalents thereof.

Detailed Description Text (63):
Other preferred therapeutic agents useful in the practice of the present invention include moieties capable of reducing or eliminating pathological proliferation, migration or hyperactivity of normal tissues. Exemplary of such therapeutic agents are those capable of reducing or eliminating hyperactivity of corneal epithelium and stroma, pathological proliferation or prolonged contraction of smooth muscle cells or pericytes of the intraocular vasculature implicated in degenerative eye disease resulting from hyperplasia or decreased vascular lumen area. Preferred agents for this purpose are TGF-beta and TGF-beta activators or production stimulators such as tamoxifen, taxol and analogs thereof, staurosporin and cytochalasin B as well as functional equivalents or derivatives thereof.

Detailed Description Text (84):
In a preferred embodiment, targeting is specific for potentially proliferating cells that result in increased smooth muscle in the intimal region of a traumatized vascular site, e.g., following angioplasty, e.g., pericytes and vascular smooth muscle cells. Aspects of the invention relate to therapeutic modalities in which the therapeutic conjugate of the invention is used to delay, reduce, or eliminate smooth muscle proliferation after angioplasty, e.g., PTCA, atheroectomy and percutaneous transluminal coronary rotational atheroblation.

Detailed Description Text (91):
In a representative example, this therapeutically effective dosage is achieved by determining in smooth muscle cell tissue culture the pericellular agent dosage, which at a continuous exposure results in a therapeutic effect between the toxic and minimal effective doses. This therapeutic level is obtained in vivo by determining the size, number and therapeutic agent concentration and release rate required for particulates infused between the smooth muscle cells of the artery wall to maintain this pericellular therapeutic dosage. The dosage form should release the therapeutic agent at a rate that approximates the pericellular dose of the following exemplary therapeutic agents: from about 0.01 to about 100 micrograms/ml nitroglycerin, from about 1.0 to about 1000 micrograms/ml of suramin, from about 0.001 to about 100 micrograms/ml for cytochalasin, and from about 0.01 to about 10.sup.5 nanograms/ml of staurosporin as well as from about 0.001 to about 100 micrograms/ml taxol.

Detailed Description Text (93):
For prevention of restenosis following angioplasty, an example of a higher trauma injury or intervention resulting in a stronger acute proliferative stimulus to smooth muscle cells, a higher dose would be required. For example, a dosing regimen is contemplated which involves a single "pre-loading" dose (or multiple, smaller pre-loading doses) given before or at the time of the intervention, with a chronic smaller (follow up) dose delivered for two to three weeks or longer following intervention. For example, a single pre-loading dose may be administered about 24 hours prior to intervention, while multiple preloading doses may be administered daily for several days prior to intervention. An exemplary single pre-loading dose is about 50 mg/kg (ranging between about 5 and about 1000 mg/kg), while an exemplary multiple pre-loading individual dose is about 10 mg/kg/day (ranging between about 0.01 and 10 mg/kg/day). Such a dosing regimen may involve a systemic pre-loading dose followed by a sustained release chronic dose, or the sustained release dosage form may be designed to deliver a large dose over a short time interval as well as a smaller chronic dose for the desired time period thereafter. Some nausea may be encountered at the higher dose; however, the use of a sustained release or other targeted dosage form is expected to obviate this side effect, because the recipient will not be subjected to a high systemic dose of the therapeutic agent.

Detailed Description Text (94):
It will be recognized by those skilled in the art that desired therapeutically effective dosages of the catheter administered sustained release dosage forms of the invention will be dependent on several factors, including, e.g.: a) the binding affinity of the binding protein associated with the dosage form, if any; b) the atmospheric pressure and duration of the infusion; c) the time over which the dosage form administered resides at the target sits d) the rate of therapeutic agent release from the particulate dosage form; e) the nature of the therapeutic agent employed; f) the nature of the trauma and/or therapy desired; and/or g) the intercellular and/or intracellular localization of the particulate dosage form. Those skilled practitioners trained to deliver drugs at therapeutically effective dosages, (e.g., by monitoring therapeutic agent levels and observing clinical effects in patients) are capable of determining the optimal dosage for an individual patient based on experience and professional judgment. In a preferred embodiment, about 0.3 atm (i.e., 300 mm of Hg) to about 3 atm of pressure applied for 15 seconds to 3 minutes to the arterial wall is adequate to achieve infiltration of a sustained release dosage form bound to the NR-AN-01 binding protein into the smooth muscle layers of a mammalian artery wall. Wolinsky et al., "Direct Intraarterial Wall Injection of Microparticles Via a Catheter: A Potential Drug Delivery Strategy Following Angioplasty," Am. Heart Jour., 122(4):1136-1140, 1991. Those skilled in the art will recognize that infiltration of a sustained release dosage form into a target cell population will probably be variable and will need to be determined on an individual basis.

Detailed Description Text (95):
It will also be recognized that the selection of a therapeutic agent that exerts its effects intracellularly, e.g., on ribosomes or DNA metabolism, will influence the dosage and time required to achieve a therapeutically effective dosage, and that this process can be modeled in vitro and in animal studies, such as those described in the Examples provided below, to find the range of concentrations over which the therapeutic conjugate or dosage form should be administered to achieve its effects of delaying, reducing or preventing restenosis following angioplasty. For example, therapeutic conjugates radiolabeled with alpha-, beta- or gamma-emitters of known specific activities (e.g., millicuries per millimole or milligram of protein) are useful for determining the therapeutically effective dosage by using them in animal studies and human trials with quantitative imaging or autoradiography of histological tissue sections to determine the concentration of therapeutic conjugate that is required by the therapeutic protocol. A therapeutically effective dosage of the therapeutic conjugate or dosage form will be reached when at least three conditions are met: namely, (1) the therapeutic conjugate or dosage form is distributed in the intimal layers of the traumatically injured vessel; (2) the therapeutic conjugate or dosage form is distributed within the desired intracellular compartment of the smooth muscle cells, i.e., that compartment necessary for the action of the therapeutic are leaser the therapeutic agent released from the dosage form extracellularly is distributed within the relevant intracellular compartment; and (3) the therapeutic agent inhibits the desired cellular activity of the vascular smooth muscle cell, e.g., proliferation, migration, increased cellular volume, matrix synthesis, cell contraction and the like described above.

Detailed Description Text (96):
It will be recognized that where the therapeutic conjugate or dosage form is to be delivered with an infusion catheter, the therapeutic dosage required to achieve the desired inhibitory activity for a therapeutic conjugate or dosage form can also be anticipated through the use of in vitro studies. In a preferred aspect, the infusion catheter may be conveniently a double balloon or quadruple balloon catheter with a permeable membrane. In one representative embodiment, a therapeutically effective dosage of a therapeutic conjugate or dosage form is useful in treating vascular trauma resulting from disease (e.g., atherosclerosis, aneurysm, or the like) or vascular surgical procedures such as angioplasty, atheroectomy, placement of a stent (e.g., in a vessel), thrombectomy, and grafting. Atheroectomy may be performed, for example, by surgical excision, ultrasound or laser treatment, or by high pressure fluid flow. Grafting may be, for example, vascular grafting using natural or synthetic materials or surgical anastomosis of vessels such as, e.g., during organ grafting. Those skilled in the art will recognize that the appropriate therapeutic

dosage for a given vascular surgical procedure (above) is determined in in vitro and in vivo animal model studies, and in human preclinical trials. In the EXAMPLES provided below, a therapeutic conjugate containing Roridin A and NR-AN-01 achieved a therapeutically effective dosage in vivo at a concentration which inhibited cellular protein synthesis in test cells in vitro by at least 5 to 50%, as judged by incorporation of radiolabeled amino acids.

Detailed Description Text (102):
It will be recognized that the conjugates and dosage forms of the invention are not restricted in use for therapy following angioplasty; rather, the usefulness of the therapeutic conjugates and dosage forms will be proscribed by their ability to inhibit cellular activities of smooth muscle cells and pericytes in the vascular wall. Thus, other aspects of the invention include therapeutic conjugates and dosage forms and protocols useful in early therapeutic intervention for reducing, delaying, or eliminating (and even reversing) atherosclerotic plaques and areas of vascular wall hypertrophy and/or hyperplasia. Therapeutic conjugates and dosage forms of the invention also find utility for early intervention in pre-atherosclerotic conditions, e.g., they are useful in patients at a high risk of developing atherosclerosis or with signs of hypertension resulting from atherosclerotic changes in vessels or vessel stenosis due to hypertrophy of the vessel wall.

Detailed Description Text (109):
2) after the passage of from about 0 to about 72 hours (preferably 24 to 72), an effective amount of a, for example, Pseudomonas exotoxin-monoclonal antibody conjugate capable of localizing to vascular smooth muscle cells is locally administered (e.g., via a catheter during an angioplasty procedure); and

Detailed Description Text (114):
2) after the passage of from about 0 to about 72 hours (preferably 24-72 hours), an effective amount of cytochalasin B is locally administered (e.g., via a catheter during an angioplasty procedure); and

Detailed Description Text (124):
Still another aspect of the present invention relates to therapeutic modalities for maintaining an expanded luminal volume following angioplasty or other vessel trauma. One embodiment of this aspect of the present invention involves administration of a therapeutic agent capable of inhibiting the ability of vascular smooth muscle cells to contract. Exemplary agents useful in the practice of this aspect of the present invention are those capable of causing a traumatized artery to lose vascular tone, such that normal vascular hydrostatic pressure (i.e., blood pressure) expands the flaccid vessel to or near to its maximal physiological diameter. Loss of vascular tone may be caused by agents that interfere with the formation or function of contractile proteins (e.g., actin, myosin, tropomyosin, caldesmon, calponin or the like). This interference can occur directly or indirectly through, for example, inhibition of calcium modulation, phosphorylation or other metabolic pathways implicated in contraction of vascular smooth muscle cells.

Detailed Description Text (125):
Inhibition of cellular contraction (i.e., loss of vascular tone) may operate through two mechanisms to reduce the degree of vascular stenosis. First, inhibition of cellular contraction for a prolonged period of time limits the number of smooth muscle cells that migrate from the tunica media into the intima, the thickening of which results in vascular luminal stenosis. Second, inhibition of cellular contraction causes the smooth muscle wall to relax and dilate under normal vascular hydrostatic pressure (i.e., blood pressure). Therapeutic agents, such as the cytochalasins, inhibit smooth muscle cell contraction without abolishing the protein synthesis necessary for traumatized, post-angioplasty or other surgically- or disease-damaged, smooth muscle cells to repair themselves. Protein synthesis is also necessary for the smooth muscle cells to secrete matrix, which fixes or retains the lumen in a state near its maximum systolic diameter as the vascular lesion stabilizes (i.e., a biologically-induced stenting effect).

Detailed Description Text (126):
This biological stenting effect not only results in an expanded vessel luminal area and increased blood flow rate through the vessel, but also significantly reduces

elastic recoil following angioplasty. Elastic recoil is an acute closure of the vessel associated with vasospasm or early relaxation of the muscular wall, due to trauma shock resulting from vessel over-stretching by a balloon catheter during angioplasty. This spasm of the tunics media which leads to decreases in the luminal area may occur within hours, days or weeks after the balloon dilation, as restoration of vascular muscle wall tone occurs. Recent observations during microscopic examination of atheroectomy specimens suggest that elastic recoil may occur in up to 25% of angioplasty procedures classified as successful, based on the initial post-procedure angiogram. Because the biological stenting procedure relaxes the artery wall following balloon angioplasty, the clinician can eliminate over-inflation and its resultant trauma shock as a means to diminish or delay the vessel spasm or elastic recoil. Reduction or elimination of over-inflation decreases trauma to the muscular wall of the vessel, thereby reducing the determinants of smooth muscle cell proliferation in the intima and, therefore, reducing the incidence or severity of restenosis.

Detailed Description Text (129):
The therapeutic agent may be targeted, but is preferably administered directly to the traumatized vessel following the angioplasty or other traumatic event. The biological stenting effect of cytochalasin B, for example, is achievable using a single infusion of the therapeutic agent into the traumatized region of the vessel wall at a dose concentration ranging from about 0.1 microgram/ml to about 1.0 micrograms/mi.

Detailed Description Text (132):
(i) retains an expanded luminal volume following angioplasty (e.g., PTCA, percutaneous transluminal angioplasty (PTA) or the like) or other trauma, including atheroectomy (e.g., rotoblater, laser and the like), coronary artery bypass procedures or the like; or resulting from vascular disease (e.g., atherosclerosis, eye diseases secondary to vascular stenosis or atrophy, cerebral vascular stenotic diseases or the like);

Detailed Description Text (138):
(i) retains an expanded luminal volume following angioplasty (e.g., PTCA, percutaneous transluminal angioplasty (PTA) or the like) or other trauma, including atheroectomy (e,g., rotoblater, laser and the like), coronary artery bypass procedures or the like; or resulting from vascular disease (e.g., atherosclerosis, eye diseases secondary to vascular stenosis or atrophy, cerebral vascular stenotic diseases or the like);

Detailed Description Text (149):
High levels of lipoprotein Lp(a) are known to constitute a major risk factor for atherosclerosis, coronary heart disease and stroke. One symptom associated with such conditions and other problems, such as restenosis following balloon angioplasty and other pathogenic conditions, is the proliferation or the migration of smooth muscle cells. No direct link between Lp(a) and proliferation of vascular smooth muscle cells had been established in the prior art.

Detailed Description Text (184):
Smooth muscle cell proliferation is a pathological factor in myocardial infarctions, atherosclerosis, thrombosis, restenosis and the like. Therapeutic agents of the present invention, including tamoxifen, TGF-beta and the like, having at least one of the activities recited above and therefore being capable of inhibiting proliferation of vascular smooth muscle cells, are useful in the prevention or treatment of these conditions. Manipulation of the proliferation modulation pathway for vascular smooth muscle cells to prevent or reduce such proliferation removes or reduces a major component of the arterial lesions of atherosclerosis and the restenosed arteries following angioplasty, for example.

Detailed Description Text (185):
More specifically, chronically maintaining an elevated level of activated TGF-beta reduces the probability of atherosclerotic lesions forming as a result of vascular smooth muscle cell proliferation. Consequently, administration of TGF-beta, TGF-beta activators or TGF-beta production stimulators protects against atherosclerosis and subsequent myocardial infarctions that are consequent to coronary artery blockage.

Also, substantially increasing the activated TGF-beta level for a short time period allows a recipient to at least partially offset the strong stimulus for vascular smooth muscle cell proliferation caused by highly traumatic injuries or procedures such as angioplasty. Continued lower dose delivery to the traumatized site further protects against restenosis resulting from vascular smooth muscle cell proliferation in the traumatized area.

Detailed Description Text (186):
Other embodiments of the present invention involve the administration of taxol or analogs thereof in soluble or sustained release dosage form. Taxol is believed to stabilize vascular smooth muscle cells against division by binding to microtubules and inhibiting the organization and ordering of the microtubule network. Cell migration may also be inhibited by this mechanism. Taxotere, an exemplary taxol analog, has a different method of action, but also inhibits cell division.

Detailed Description Text (242):
While this type of non-specific inhibition was judged to be of potential value for biological atheroectomy, it was not considered desirable for treatment of restenosis following angioplasty where dead and dying cells may release factors that stimulate smooth muscle proliferation.

Detailed Description Text (266):
The therapeutic conjugates of the invention are useful for inhibiting stenosis following vascular trauma or disease. In an illustrative example, vascular trauma that is induced during angioplasty is treated during the surgical procedure by removing the catheter used to perform the angioplasty, and inserting a balloon infusion catheter into the vessel. The infusion catheter is positioned with the instillation port (or, alternatively, a permeable membrane region) in the traumatized area of the vessel, and then pressure is applied to introduce the therapeutic conjugate. For example, an infusion catheter with two balloons may be used, and when one balloon is inflated on either side of the trauma site a fluid space is created that can be filled with a suitable infusion fluid containing the therapeutic conjugate. It has been reported previously that infusion of a horseradish peroxidase (HRP) marker enzyme at a pressure of 300 mm Hg over 45 seconds in dog or human coronary arteries resulted in penetration of the HRP into the vessel wall (6). However, HRP is a smaller molecule than NR-AN-01 and human and dog coronary arteries are also considerably smaller than the carotid or femoral arteries in the present domestic pig model system. Experiments were therefore conducted to determine, in a domestic pig model system, the infusion conditions suitable for delivery of a therapeutic conjugate to the vascular smooth muscle cells in carotid and femoral arteries. Delivery conditions were monitored by evaluating the penetration of the therapeutic conjugate into the vascular wall, and specific binding of the therapeutic conjugate to the vascular smooth muscle cells in the vessel wall.

Detailed Description Text (272):
Intimal smooth muscle proliferation that follows balloon catheter-induced trauma is a good model to evaluate the therapeutic efficacy of conjugates for inhibiting smooth muscle cell activity in vivo in response to vascular trauma, including restenosis following angioplasty. Domestic pigs were used to study the effects of NR-AN-01 (i.e., termed vascular smooth muscle binding protein or simply VSMBP in these studies; and therapeutic conjugates with Roridin A are termed VSMBP-RA). The events which normally follow balloon angioplasty in the porcine artery have been described previously (12). In these studies, dilation of the carotid artery using an oversized balloon (balloon: artery ratio approximately 1.5:1) resulted in complete endothelial denudation over an area of 1.5-2 cm in length. Although this length of traumatic injury was selected in an attempt to minimize thrombosis, there was still marked platelet deposition and thrombus formation. The procedure also resulted in dissection through the internal elastic lamina into the arterial media and necrosis of medial smooth muscle cells. Intimal thickening due to smooth muscle proliferation was apparent 7 days after injury and reached a mean maximum thickness of 85 mm at 14 days. The histological appearance of this neointima is very similar to the proliferative neointimal tissue of human restenosis (13).

Detailed Description Text (283):

The results presented in FIG. 9A show (at 160.times.magnification) a cross-sectional of an untreated artery 5 weeks after angioplasty. Dominant histological features of the artery include displacement of the endothelium (see #1 in FIG. 9A) away from the internal elastic lamina (see #2, FIG. 9A), apparently due to intimal smooth muscle proliferation (see #3, FIG. 9A).

Detailed Description Text (284):
The results presented in FIG. 9B show (at 160.times.magnification) a cross-section of a treated artery 5 weeks after angioplasty and infusion of the RA-NR-AN-01 therapeutic conjugate. The vessel in this section was subjected to greater mechanical stresses than the vessel shown in FIG. 9A, with multiple sites where the external elastic membrane was ruptured and associated proliferation of smooth muscle cells in the outer layers of the media was observed (i.e., see #4 in FIG. 9B). Treatment with therapeutic conjugate inhibited intimal hypertrophy, as evidenced by the lack of displacement of the endothelium (see #1, FIG. 9B) from the internal elastic lamina (see #2, FIG. 9B). Surprisingly, this inhibitory effect on intimal smooth muscle cells was accomplished without inhibiting hypertrophy of medial smooth muscle cells in the areas where the external elastic membrane was ruptured (see #4, FIG. 9B).

Detailed Description Text (287):
In FIG. 9B, therapeutic conjugate administered at the site following angioplasty resulted in approximately 95% inhibition of the smooth muscle hypertrophy that restricted the lumen of the untreated vessel (FIG. 9A). Significantly, the therapeutic conjugate exerted its effects on the smooth muscle cells migrating from the medial smooth muscle layers into the intima, without affecting either endothelium, or producing any signs of necrosis (i.e., cell death) in the smooth muscle cells in the medial layers of the arterial wall. Studies also failed to show any histological signs of mononuclear infiltration or fibrosis such as might result from toxic effects on the vessel wall. Also, visible signs of healing were observed in the intimal layers of treated vessels and with re-growth of endothelium observed, i.e., endothelial cells growing over the thin layer of smooth muscle cells in the intima that lie between the endothelium and internal elastic lamina (i.e., #1 and #2, FIG. 9B). These combined histological observations suggest the highly desirable features of wound healing, re-growth of endothelium and improved vascular strength following treatment with a therapeutic conjugate that inhibits smooth muscle hyperplasia in the intimal layers of the vessel.

Detailed Description Text (391):
A cytochalasin B dose response study was then conducted on 10 pigs, following the experimental protocol described in Example 7. Briefly, both arteries in each of 2 pigs were treated with one of the following doses of cytochalasin B: 0.0 .mu.g/ml (i.e., PBS negative control); 0.01 .mu.g/ml; 0.10 .mu.g/ml; 1.0 .mu.g/ml; and 10.0 .mu.g/ml. The agent was delivered by intraluminal catheter at 1 atm pressure for 3 min, and the arteries were evaluated 3 weeks later by the morphometric analysis system described above. The ratio of treated artery luminal area to proximal normal artery luminal area was determined as a percent change in treated vs. normal area. A significant threshold effect was observed at doses from 0.1 .mu.g/ml (.apprxeq.140% increase) to 1.0 .mu.g/ml (FIG. 14). The 10 .mu.g/ml dose appeared to be toxic to the vascular smooth muscle cells (data not shown). The subthreshold dose (0.01 .mu.g/ml) and negative control (PBS) exhibited a .+-..apprxeq.20% change in luminal area. These data suggest that cytochalasin B acts as a "biological stent" when delivered to traumatized arteries.

Detailed Description Text (430):
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2. Fanelli, C. et al. 1990. Restenosis following coronary angioplasty. Amer. Heart Jour. 119: 357-368.

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12. Steele P. M., Chesebro J. H., Stanson A. W., et al. 1985. Balloon angioplasty: natural history of the pathophysiological response to injury in a pig model. Circ. Res. 57:105-112.

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13. Schwartz, R. S., Murphy J. G., Edwards W. D., Camrud A. R., Vliestra R. E., Holmes D. R. Restenosis after balloon angioplasty. A practical proliferative model in porcine coronary arteries. Circulation 1990; 82:2190-2200.

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Chapman, G.D., et al., "A Bioabsorbable Stent: Initial Experimental Results", Supplement III Circulation, 82, p. III-72, Abstract No.0283 Oct. 1990).

Other Reference Publication (27):
Detre, K., et al., "Percutaneous Transluminal Coronary Angioplasty in 1985-1986 and 1977-1981", New England J. Med., 318, 265-270 (1988).

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Other Reference Publication (46):
Hanke, Hartmut, Md, et al., "Inhibition of Cellular Proliferation After Experimental Balloon Angioplasty by Low-Molecular-Weight Heparin", Circulation, 85, 1548-56 (Apr. 1992).

Other Reference Publication (60):
Lafont, et al., "Post-Angioplasty Restenosis in the Atherosclerotic Rabbit: Proliferative Response or Chronic Constriction", Circulation, 88, p. I-521, Abstract No. 2806 (1993).

CLAIMS:

1. A therapeutic method comprising inhibiting stenosis or restenosis of a blood vessel by administering to a mammal an effective amount of taxol or a structural analog thereof.

2. The method of claim 1 wherein an effective amount of taxol is administered.

9. The method of claim 8 wherein the vascular trauma is associated with angioplasty, placement of a stent, or grafting.

13. A method for biologically stenting a mammalian blood vessel which is subjected to procedural vascular trauma, which method comprises administering to the blood vessel an amount of taxol or a structural analog thereof effective to biologically stent the vessel.

17. The method of claim 13 wherein the vessel is subjected to angioplasty, placement of a stent, or grafting.

19. The method of claim 13 wherein taxol is administered.

21. A therapeutic method comprising:

(a) administering to a blood vessel of a mammal traumatized by a surgical procedure an amount of taxol or a structural analog thereof effective to biologically stent the vessel; and

(b) administering an amount of a sustained release dosage form comprising an amount of a cytostatic agent effective to inhibit proliferation of the cells of the vessel caused by said procedure.

24. The method of claim 21 wherein the cytostatic agent comprises taxol or a

structural analog thereof.

26. The method of claim 21 wherein the administration of taxol or a structural analog thereof is before, during or after the trauma.

27. The method of claim 26 wherein the administration of taxol or a structural analog thereof and the administration of the cytostatic agent is simultaneous.